

Project Report: Microbial symbionts: Agents for reorganizing genome architectures.

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Project Progress

Bacterial endosymbionts, intracellular microbes that replicate within the domain of the host cell, have catalyzed the origin of the eukaryotic cell, the evolution of multicellular complexity, and diversification of numerous eukaryotic lineages. Mitochondria and chloroplasts, for example, result from endosymbiotic events of lasting significance that fundamentally changed cellular structure, species diversity, and the range of acceptable habitats of Life. Today, endosymbionts include several important human pathogens as well as diverse mutualistic lineages that are critical to the reproduction and success of their eukaryotic hosts. This project targets associations between bacteria and insects as model systems to study the drivers and consequences of genome remodeling.

During the reporting period, we made significant progress toward understanding the impact of long-term, stable genome interactions on molecular evolution and genome architecture. Our extensive molecular evolutionary analysis of *Blochmannia* and its ant hosts demonstrated strict host-symbiont cospeciation, and revealed extremely rapid evolutionary rates indicating unprecedented mutation rates in this ant mutualist (Degnan et al. 2004). As an extension of this work, we have also completed a phylogenetic analysis of endosymbiotic and free-living bacteria that explicitly accounts for their wide variation in GC content and shows independent origins of primary endosymbionts (Herbeck et al., submitted). We have published two population genetic studies showing exceptionally strong effects of mutational pressure and genetic drift in endosymbionts (Herbeck et al. 2003a; Wernegreen and Funk, in press). In addition, we published a genome-wide analysis of codon and amino acid usage in *Wigglesworthia*, the long-term obligate bacterial endosymbiont of the tsetse fly (Herbeck et al. 2003b). We used multivariate statistical analyses to test the hypothesis that mutational bias and genetic drift shape synonymous codon usage and amino acid usage in this severely reduced, 697-kb genome. Our results showed that codon and amino acid usage in *Wigglesworthia* reflect a strong AT mutational bias and elevated levels of genetic drift, consistent with expected effects of an endosymbiotic lifestyle and repeated population bottlenecks. However, these impacts of mutation and drift are apparently attenuated by selection on amino acid composition at functionally conserved, high-expression genes.

Our research on *Wolbachia*, a reproductive parasite, explores the evolution of a dynamic genome–genome interaction. We demonstrated significant genome size variability among parasitic *Wolbachia* strains (Bordenstein and Wernegreen, in prep). This result sets the stage for comparative genomic analyses to identify changes in gene content that trigger new host associations and symbiont phenotypes. We also documented recombination of an active bacteriophage in *Wolbachia* strains, and developed a novel hypothesis on the exchange of genetic information between intracellular microbial communities (Bordenstein and Wernegreen, in press). This research is part of the NAI/NRC Fellowship of Dr. Seth Bordenstein, and is described in more detail in his separate report.

Highlights

- Bacterial endosymbionts of diverse insects show similar patterns of genome reduction, strong AT mutational bias, and effects of genetic drift on protein evolution. However, certain distinct features of these endosymbiont genomes (e.g., the specific biosynthetic genes they retain) can be linked to the nutritional physiology of their specific hosts.
- Parasitic *Wolbachia* strains show considerable genome size variation that may underlie their distinct host ranges and symbiont phenotypes.
- Bacteriophages of *Wolbachia* play a significant role in the genome flux of this endosymbiont. These mobile elements are widespread among *Wolbachia*, transfer laterally between divergent strains, and may shuttle chromosomal genes.

Roadmap Objectives

- **Objective No. 4.2:** Foundations of complex life
- **Objective No. 5.1:** Environment–dependent, molecular evolution in microorganisms
- **Objective No. 5.2:** Co–evolution of microbial communities
- **Objective No. 6.2:** Adaptation and evolution of life beyond Earth